Journal of Toxicology and Environmental Health, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713667303

Estrogenicity of Organophosphorus and Pyrethroid Pesticides

Haiyan Chen [a]; Jigao Xiao [b]; Gang Hu [b]; Jianwei Zhou [a]; Hang Xiao [a];Xinru Wang [a]
[a] Institute of Toxicology, Nanjing Medical University, Nanjing, People's Republic of China. [b] Institute of Basic Medicine Sciences, Nanjing Medical University, Nanjing, People's Republic of China.

To cite this Article Chen, Haiyan , Xiao, Jigao , Hu, Gang , Zhou, Jianwei , Xiao, Hang andWang, Xinru(2002) 'Estrogenicity of Organophosphorus and Pyrethroid Pesticides', Journal of Toxicology and Environmental Health, Part A, 65: 19, 1419 — 1435

To link to this Article: DOI: 10.1080/00984100290071243

URL: http://dx.doi.org/10.1080/00984100290071243

Journal of Toxicology and Environmental Health, Part A, 65:1419–1435, 2002

1528-7394/02 $12.00 + .00
DOI: 10.1080/0098410029007124 3

ESTROGENICITY OF ORGANOPHOSPHORUS AND PYRETHROID PESTICIDES

Haiyan Chen

Institute of Toxicology, Nanjing Medical University, Nanjing, People's Republic of China

Jigao Xiao, Gang Hu

Institute of Basic Medicine Sciences, Nanjing Medical University, Nanjing, People's Republic of China

Jianwei Zhou, Hang Xiao, Xinru Wang

Institute of Toxicology, Nanjing Medical University, Nanjing, People's Republic of China

Although organophosphorus and pyrethroid pesticides are considered environmental contaminants, their estrogenic potentials are still ubiquitous and unclear. The present study was undertaken to evaluate the estrogenic activities of nine pesticides (phoxim, malathion, monocrotophos, dimethoate, opunal, fenvalerate, cypermethrin, permethrin, and deltamethrin) using three in vitro methods [E-Screen assay, estrogen receptor (ER) competitive binding assay, and pS2 expression assay]. All the pyrethroid pesticides tested induced MCF-7 cell proliferation significantly, while organophosphorus pesticides did not. The estrogenic potency were ranked as permethrin > fenvalerate > cypermethrin > deltamethrin. The proliferation induced by cypermethrin, permethrin, and deltamethrin was blocked by ICI 182.780, while fenvalerate only partly inhibited it. In addition, pyrethroid pesticides inhibited the binding of [^{3}H]estradiol to ER, while the organophosphorus failed to do so. Fenvalerate, permethrin, and cypermethrin induced pS2 mRNA expression with varying potency, while there were no significant effects in deltamethrin-treated groups. Our findings provide evidence to support the idea that pyrethroid pesticides tested produce an ER-specific, agonist response. Fenvalerate induced MCF-7 cell proliferation by a mechanism not involving ER-mediated pathway. Organophosphorus pesticides tested showed no estrogenic potential. Compared with the pS2 expression assay, E-Screen was a more sensitive and useful assay for screening of the xenoestrogenic chemicals.

Pesticides are commonly used for the control of agricultural and indoor pests (Garey & Wolff, 1998). The ubiquitous nature of pesticide usage with minimal precautions has resulted in contamination of food, the workplace, and the environment (Aprea et al., 1998; Cole et al., 1998). Organophosphorus and pyrethroid pesticides are the most popular pesticides used in

Received 20 August 2001; accepted 18 October 2001.

The authors thank Dr. Changkun Chen for presenting the pesticides and Dr. John McLachlan and Dr. Melnik for providing the protocol of making CDS-FBS. This work was supported by grants from a Preliminary Study of the Greatest Special Project in the National Basic Research (200150), National "95" Key Project (969060411), and National Pan-Deng Project (2000504), People's Republic of China.

Address correspondence to Xinru Wang, Institute of Toxicology, Nanjing Medical University, 140 Hanzhong Road, Nanjing, 210029, Jiangsu, P.R. China. E-mail: xrwang@njmu.edu.cn

China. Organophosphorus pesticides exert their effects by phosphorylation of acetylcholinesterase enzyme activity, causing accumulation of acetylcholine, overstimulation of cholinergic receptors, and consequent clinical signs of toxicity (Pope, 1999; Abu-Qare et al., 2001; Mileson et al., 1998). There is currently concern that some chemicals present in the environment, workplace, and home (including pesticides, alkylphenols, phthalates, polychlorinated biphenyl, antioxidants, and *o-p′*-DDT) may interact with the estrogen receptor (ER) in mammalian cells and thereby produce adverse reproductive and developmental effects (Sonnenschein & Soto, 1998; McLachlan, 1993; Tonilol et al., 1995). Recent reports showed that pyrethroid pesticides (*d-trans*-allethrin, fenvalerate, sumithrin, and permethrin) exerted estrogenic and antiprogestagenic activities in MCF-7 human breast carcinoma cell lines (Garey & Wolff, 1998; Go et al., 1999). Fenvalerate significantly increased alkaline phosphatase activity in Ishikawa Var-I human endometrial cancer cells, and pS2 mRNA expression and cell proliferation in MCF-7 cells. However, the increases were not consistently inhibited by estrogen receptor antagonist ICI 164.384. Significant increases in alkaline phosphatase activity and pS2 expression were also found with sumithrin exposure, whereas no significant change in MCF-7 cell proliferation occurred. It was reported that none of the pyrethroid pesticides (*d-trans*-allethrin, cypermethrin, empenthrin, fenvalerate, imiprothrin, *d*-phenothrin, prallethrin) produced significant estrogenic or antiestrogenic effects at concentrations ranging from 100 n*M* to 10 μ*M* (Saito et al., 2000). Pyrethroid insecticides thus do not appear to affect the classic hERα-mediated activation pathway in vitro. The estrogenicity of environmental chemicals has been documented by the production of estrogen-specific effects in many systems (Roy et al., 1997; Rawlings et al., 1998). The estrogenic effects include (1) increase in uterine weight and enzyme activities (Gray et al., 1997), (2) changed production of vitellogenin (Petit et al., 1997), a female-specific yolk protein in male fish, (3) stimulation of cell proliferation (Mellanen et al., 1996), and (4) elevated transcription of estrogen responsive genes in human cancer cells (Wang et al., 1997; Jorgensen et al., 2000; Petit et al., 1997). The estrogenic activity of many chemicals is based on their interactions with ER, suggesting that assays measuring the activity of hormones receptors in response to environmental chemicals will provide insight into the hormonal or antihormonal activities of chemicals (Klotz et al., 1997; Carney et al., 1997). In order to verify whether organophosphorus and pyrethroid pesticides in vitro have the ability to interact with ER, nine most popularly used pesticides (five organophosphorus and four pyrethroid pesticides) in China were examined. Further, the effects of pyrethroid pesticides on mRNA levels for the estrogen-sensitive pS2 gene by reverse-transcription polymerase chain reaction (RT-PCR) were determined.

MATERIALS AND METHODS

Cell Line and Cell Culture Conditions

Human breast cancer estrogen-sensitive MCF-7 cells were obtained from Shanghai Institute for Cell Science, Chinese Academy of Science. For routine maintenance, cells were grown in RPMI 1640 (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) at 37°C in an atmosphere of 5% CO_2/95% air under saturating humidity and passaged every week (1:4 to 1:5 split) by trypsinization with 0.25% trypsin/0.02% ethylenediamine tetraacetic acid (EDTA) disodium salt solution (Life Technologies, Paisley, UK).

Chemical Tested

All the tested pesticides were obtained by Dr. CangKun Chen (Nanjing Agricultural University, Nanjing, China). The purity of all the pesticides tested was above 90%. Test chemicals were dissolved in 100% ethanol or dimethyl sulfoxide (DMSO) at the highest concentration possible. Stock solutions were then subsequently diluted in ethanol or DMSO in the assays.

Removal of Sex Steroids by Charcoal-Dextran-Stripped Fetal Bovine Serum (CDS-FBS)

This method is based on the protocol provided by Dr. John McLachlan and Dr. Lilia Melnik (Environmental Endocrinology Laboratory, Center for Bioenvironmental Research, Tulane School of Medicine). A 0.25% charcoal (Sigma Chemical Co., St. Louis, MO)–0.025% dextran (Sigma Chemical Co., St. Louis, MO) suspension was prepared and autoclaved for 25 min. Charcoal-dextran (CD) suspension aliquots of a volume similar to the serum aliquots to be processed were centrifuged at 800 × g for 15 min. Supernatants were aspirated and serum aliquots were mixed with the charcoal pellets. This charcoal–serum mixture was maintained in suspension by vortexing and shaking for 30 min. This suspension was centrifuged at 3000 × g for 30 min. The supernatant was removed, mixed with charcoal, and the former steps are repeated. The supernatant (CDS-FBS) was then transferred and stored at –20°C until needed.

The E-Screen Assay

The experimental procedures were based on previously described methods with some modifications (Villalobos et al., 1995). Briefly, MCF-7 cells were trypsinized and plated into 96-well plates with the medium (5% CDS-FBS supplemented to phenol red-free RPMI 1640) at an initial concentration of 10^3 cells per well. Cells were allowed to attach for 24 h; then the medium was removed and replaced by the medium containing a range of concentration of test compounds. The bioassay was terminated on day 6 (late exponential phase) by removing the medium from the wells, adding a thiazolyl blue (Sigma Chemical Co., St. Louis, MO) solution (5 mg/ml PBS buffer). After 4 h, the solution was removed and 200 μl DMSO/well was added; after 10 min, the absorbance was determined at 490 nm by CERES900 (Bio-Tek Instruments, Inc., Winooski, VT). Fold proliferation of each exposure groups was determined as the ratio of group absorbance to control absorbance.

Uterine Cytosol (Estrogen Receptor) Preparation

Uterine cytosol was prepared as described previously with some modifications (Blair et al., 2000). Adult female Sprague-Dawley rats (n = 15, 180–

200 g, SIPPR/BK, certificate 02-49-2) were housed in an air-conditioned room with controlled lighting conditions (12-h light/dark) and allowed free access to water and rat feed. After sacrifice by decapitation, uteri were excised, trimmed of excess fat and mesentery, weighed, and stored in liquid nitrogen. After all the rats were sacrificed, the pooled uteri were placed in fresh, ice-cold TEDGSP buffer (10 m*M* Tris, 1 m*M* EDTA, 2 m*M* dithiothreitol, 10% glycerol, 10 m*M* sodium molybdate, 1 m*M* phenylmethylsulfonyl fluoride (pH 7.5) at a concentration of 1 g tissue/6 ml buffer. After precooling (4°C) the homogenization probe, uterine tissue was homogenized at 4°C with a Polytron homogenizer (Brinkmann Instruments, Inc., Westbury, NY) using 5-s bursts. The resulting homogenate was transferred to precooled (4°C) centrifuge tubes and centrifuged at 800 × g for 15 min at 4°C; then the supernatant was transferred into precooled (4°C) ultracentrifuge tubes and centrifuged at 105,000 × g for 60 min at 4°C. The resultant supernatant was used as cytosolic fraction, and protein content was determined by the method of Lowry et al. (1951). The solution was decanted and stored at –70°C until used in the competitive binding assay.

Estrogen Receptor Competitive-Binding Assay

Analytical procedures followed previously described methods with some modifications (Blair et al., 2000). Briefly, 10^{-9} *M* 2,3,6,7-[^{3}H]estradiol (Amersham Pharmacia, Little Chalfont, Bucks., UK) was incubated with 20 µl of increasing concentrations of radioinert competitor, 100 µl of uterine cytosol preparation, and 60 µl of TEDGSP buffer. Reaction mixtures were incubated at 4°C for 16 h. Following the incubation period, 200 µl of cold dextran–charcoal buffer [0.25% dextran (Sigma Chemical Co., St. Louis, MO) and 2.5% activated charcoal (Sigma Chemical Co., St. Louis, MO) in TE buffer (10 m*M* Tris, 1 m*M* EDTA, pH 8.0)] was added to each tube to separate the bound ligand from the free ligand. These tubes were incubated in an ice-water bath for 30 min and vortexed for 10 s at 10-min intervals. Tubes were subsequently centrifuged (800 × g) at 4°C for 5 min. The supernatant was decanted into vials containing 5 ml of scintillation cocktail. Radioactivity was measured on a Packbeta LKB1219 liquid scintillation analyzer (Packard Instrument Co., Meriden, CT). In addition to the radioinert competitors, each assay incubated a control tube (no competitor added; represented total binding of [^{3}H]estradiol ([^{3}H]-E_2); averaged approximately 15,000 dpm) and an 17β-estradiol (E_2) standard curve (1×10^{-4}, 1×10^{-5}, 1×10^{-6}, 1×10^{-7}, 1×10^{-8}, 1×10^{-9}, 1×10^{-10}, 1×10^{-11} or 1×10^{-12} *M* concentration) for quantity control purposes. The 1×10^{-7} *M* E_2 tube contained a 100-fold molar excess of radioinert E_2 compared to [^{3}H]-E_2 and thus represented nonspecific binding (NSB; averaged approximately 800 dpm). Radioactivity counts (dpm) of the NSB were subtracted prior to calculation of percent [^{3}H]-E_2 bound. Data for each competitor and the E_2 standard curve were plotted as percent [^{3}H]-E_2 bound versus molar concentration, and the IC50 (the concentration of chemical necessary to inhibit the binding of [^{3}H]-E_2 to ER by 50%) for each competitor was determined.

The pS2 mRNA Assay

MCF-7 cells were trypsinized and plated into 6-well plates with the medium (5% CDS-FBS supplemented to phenol red-free RPMI 1640). As in the E-Screen assay, after 24 h of preincubation, pesticides or E_2 of variant concentrations were used to treat the cells. Ethanol (0.1%) or DMSO was added to the control. All cells were harvested at the same time. The total RNA was purified with TRIzol (Biobasic, Inc., Ontario, Canada) reagent according to the manufacturers protocol. The lysed cells were frozen in TRIzol at –80°C. After they were thawed at room temperature, chloroform (0.2 ml) was added to each sample and vortexed. The mixture was centrifuged at 12,000 × g for 15 min at 4°C. The aqueous phase (0.6 ml) was transferred to an RNase-free tube and 0.3 ml isopropyl alcohol was added. The mixture was incubated at room temperature for 10 min and centrifuged for 10 min at 12,000 × g at 4°C. The supernate was removed, and the RNA pellet was washed once with 75% ethanol and centrifuged at 7500 × g for 5 min at 4°C. After it was air-dried for 10 min, the RNA pellet was dissolved in 20 μl of RNase-free water. The RNA concentration was adjusted to 0.6 μg/μl and used for RT-PCR. In a sterile, nuclease-free microcentrifuge tube, 1 μg oligo-d(T)$_{18}$AGC was added to the 2 μg RNA sample in a total volume of 13.5 μl and heated to 70°C for 5 min. The tubes were chilled on ice for 5 min and centrifuged to collect the solution. The following components were added in the order shown: AMV RT 5 × reaction buffer 5 μl, 10 m*M* dNTPs 2.5 μl, RNasin 10 U, AMV reverse transcriptase (RT; Promega Co., Madison, WI) 10 U, and nuclease-free water (up to 25 μl). The RT reaction mixture was incubated for 1 cycle at 42°C for 60 min, 70°C for 5 min, and 4°C for 5 min. The entire cDNA complement produced during RT reaction was used in subsequent PCRs. All the primers used in this study were described by Gillesby and Zacharewski (1999) and purchased from Sheng Gong Biomedicine Co. (Shanghai, China) (see Table 1). The PCR mixture contained RT product 2 μl, 10 × PCR buffer 5 μ1 (pH = 7.6), 50 p*M* forward and reverse primer, 1.5 m*M* $MgCl_2$, 0.1 m*M* dNTPs, and Taq DNA Polymerase (Promega Co., Madison, WI) 2 U (total volume 50 μl). The reaction condition was 95°C for 5 min, and it continued for 30 cycles of 94°C for 45 s, 58°C for 45 s, and 72°C for 45 s (for pS2) and 94°C for 45 s, 66°C for 45 s, and 72°C for 45 s (for β-actin), followed by final template extension at 70°C for 7 min. After completion of the reactions, PCR products were separated in 1.5% agarose gel containing 0.1% ethidium bromide. The net intensity of the bands, corresponding to pS2 and β-actin, were analyzed with a Kodak digital identification (ID) imagine analysis software (Eastman Kodak Co., Rochester, NY). The pS2 concentration was determined by normalizing the pS2 net intensity to the β-actin net intensity.

TABLE 1. Primers Used for Amplification of Target mRNA in RT-PCR Reaction

Gene product	Forward primer (5′-3′)	Reverse primer (5′-3′)
pS2	ttt gga gca gag agg agg caa tgg	tgg tat tag gat aga agc acc agg g
β-Actin	gca cca cac ctt cta caa tga gc	gac gta gca cag ctt ctc ctt aat g

Statistics

Statistical analysis was performed by analysis of variance (ANOVA). The criterion for significance was set at $p < .05$.

RESULTS

E-Screen Assay

Addition of E_2 to CDS-FBS-supplemented medium increased the number of MCF-7 cells in the culture. Maximum proliferative effect was obtained at concentration of 1 n*M* E_2 and higher (Figure 1). Cell yields were threefold greater than in control (0.1% ethanol) culture after 6 d. In the absence of E_2 (control), cells proliferated minimally. Thus 1 n*M* E_2 was selected as the positive control. Concentration responses to five organophosphorus pesticides are shown in Figure 1. There are no significant effects on cell proliferation after exposure to these chemicals. Figure 2 illustrates the promotion of cell growth by four pyrethroid pesticides. The PE (proliferation effect) values for fenvalerate, cypermethrin, deltamethrin, and permethrin are 2.17, 1.46, 1.74, and 2.69, respectively. In comparison with the PE of E_2 (3.46), all the pyrethroid pesticides showed a partial agonistic response. In addition, estimated RPP (relative proliferate potency, PE ratios of chemical to E_2) allowed us to rank the pyrethroid pesticides by their estrogenic potency: E_2 > permethrin > fenvalerate > cypermethrin > deltamethrin.

ER Competitive-Binding Assay

Radioinert 17β-estradiol effectively competed with $[^3H]$-E_2 for binding to ER at the concentrations tested (Figure 3). Of the four pyrethroid pesticides, fenvalerate and cypermethrin were the most effective pesticides at inhibiting the binding of $[^3H]$-E_2 to ER (Figure 4). The IC50 were 0.479 and 0.562 m*M*, respectively; while permethrin and deltamethrin inhibit the binding of $[^3H]$-E_2 to ER, it was less than 67%. Organophosphorus pesticides did not compete significantly with $[^3H]$-E_2 for binding to ER at any concentrations tested.

pS2 Expression Assay

At 10^{-7} *M*, 17β-estradiol induced pS2 mRNA expression after a 2-h exposure; the highest level was at 6 h, and expression remained elevated through the 48-h point (Figure 5). Thus 6 h was selected as the exposure time in the following experiments. As shown in Figure 6, pyrethroid pesti-

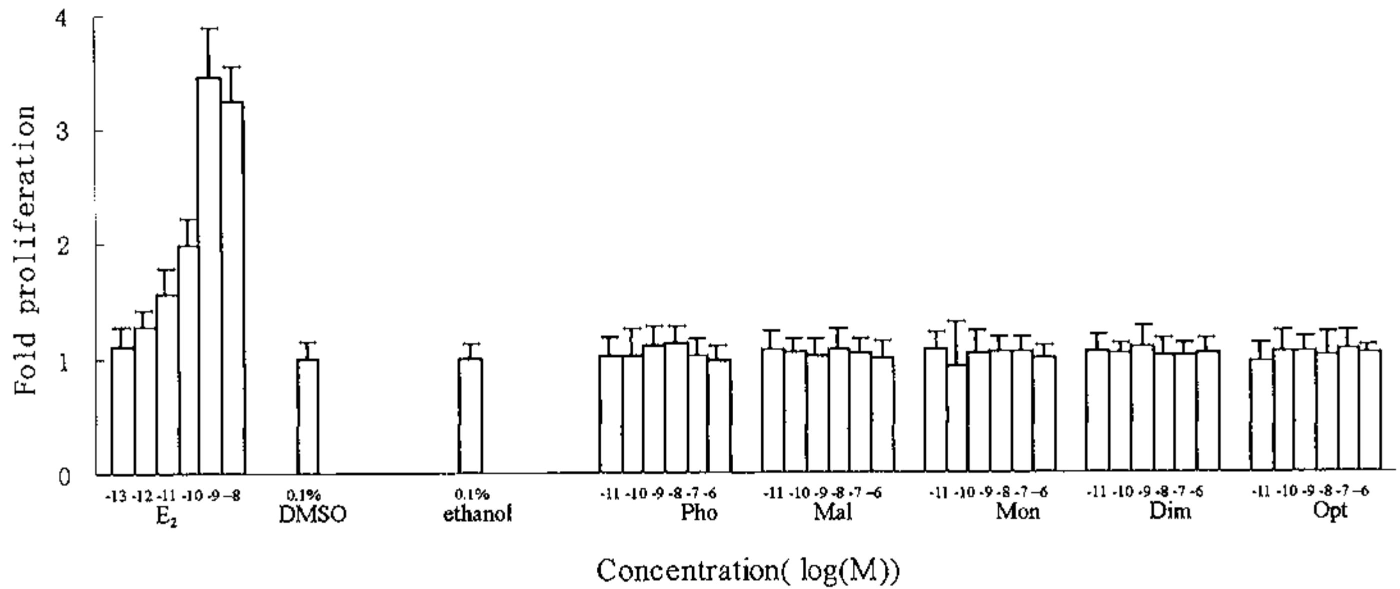

FIGURE 1. Proliferation of MCF-7 cells growth in 5% CDS-FBS-supplemented medium exposed for 144 h to E_2 and organophosphorus pesticides. E_2, 17β-estradiol; Pho, phoxim; Mal, malathion; Mon, monocrotophos; Dim, dimethoate; Opt, optunal. Results are presented as mean ± SD of six separate determinations.

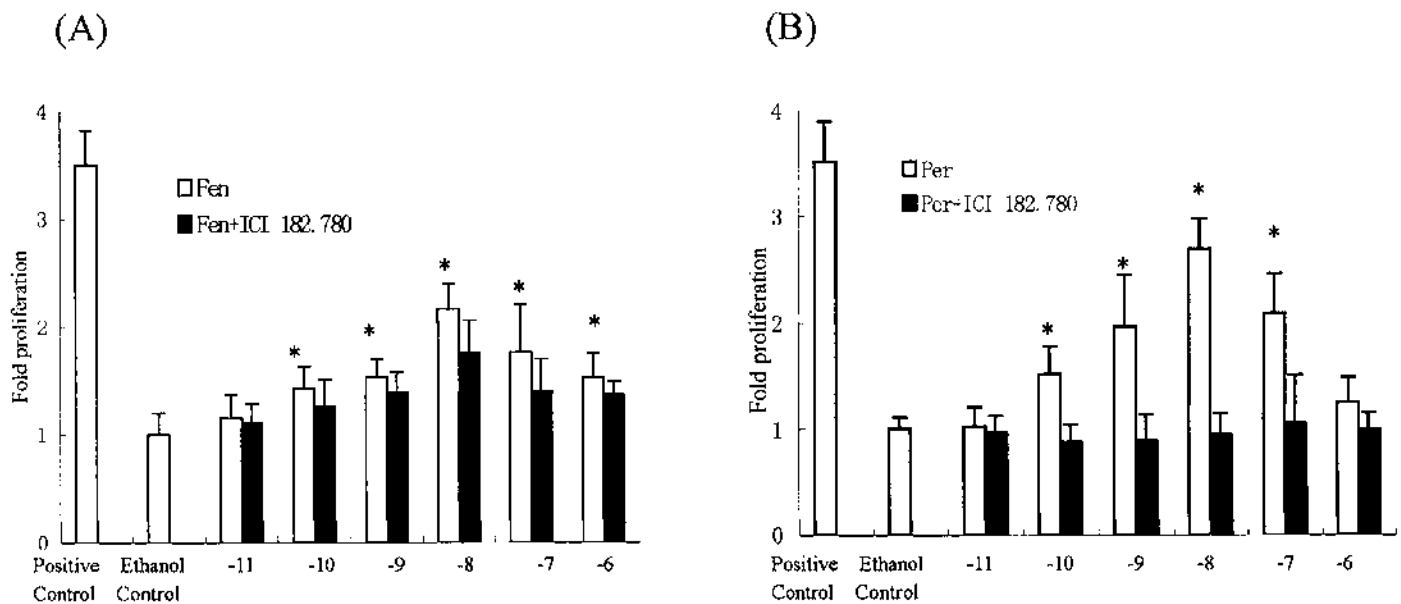

FIGURE 2. Proliferation of MCF-7 cells growth in 5% CD-FBS-supplemented medium exposed for 144 h to E_2 and pyrethroid pesticides. positive control, 1 n*M* 17β-estradiol; Fen, fenvalerate; Per, permethrin; Del, deltamethrin; Cyp, cypermethrin. Results are presented as mean ± SD of six separate experiments. Asterisk indicates significantly different from control ($p < .05$).

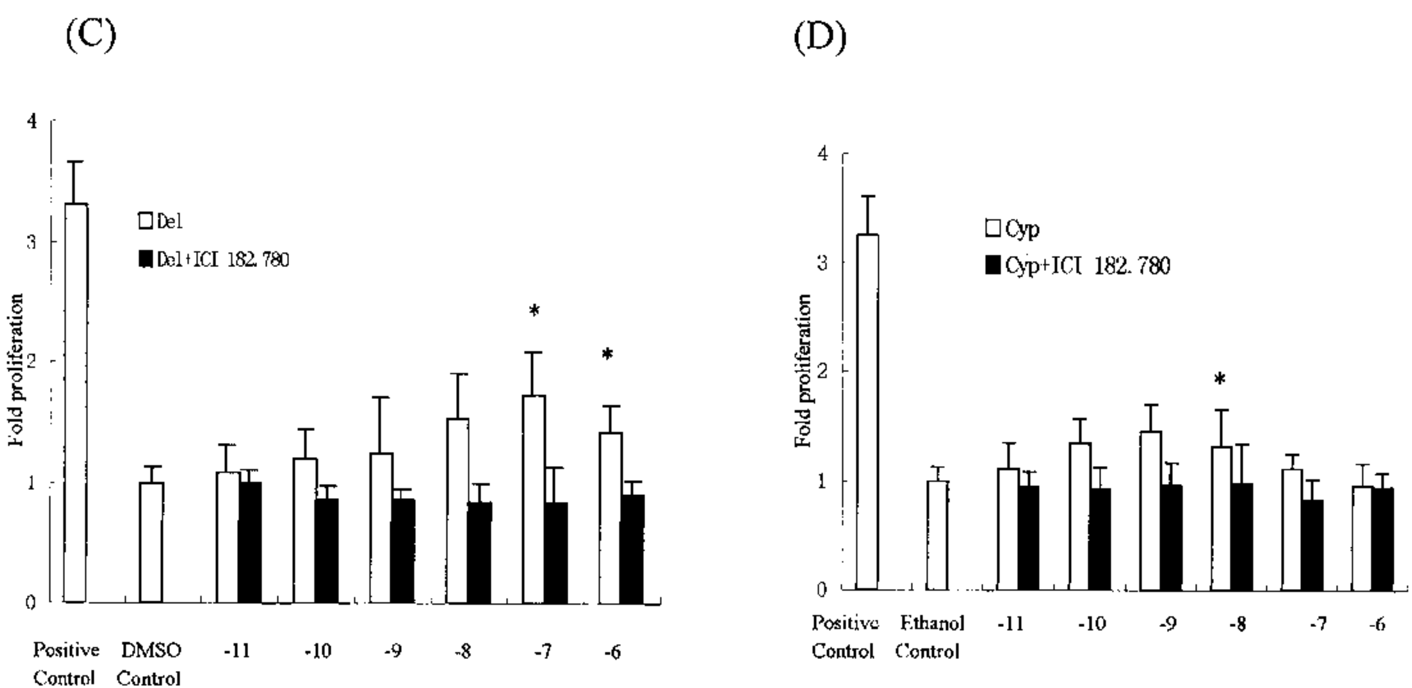

FIGURE 2. (*Continued*) Proliferation of MCF-7 cells growth in 5% CD-FBS-supplemented medium exposed for 144 h to E_2 and pyrethroid pesticides. positive control, 1 n*M* 17β-estradiol; Fen, fenvalerate; Per, permethrin; Del, deltamethrin; Cyp, cypermethrin. Results are presented as mean ± SD of six separate experiments. Asterisk indicates significantly different from control ($p < .05$).

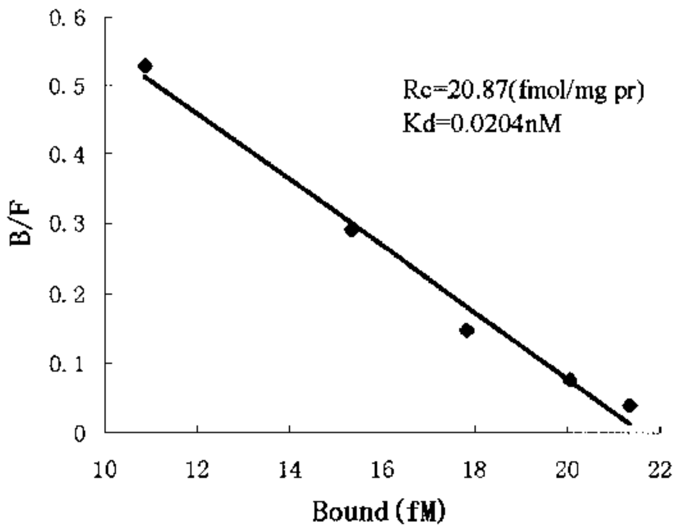

FIGURE 3. Schatchard analysis of estrogen receptor derived from rat uteri. The K_d value was calculated from the scope of the plot. The concentrations of radioactive estradiol used for ER assay were 0.05, 0.1, 0.2, 0.4, and 0.8 n*M*. Data obtained from each concentration were averages of two determinations. B/F, bound/free; Rc, receptor contents.

cides increased the level of pS2 mRNA to a comparable extent. The pS2 mRNA expression increased 1.67-, 1.28-, and 2.01-fold after 10^{-6} *M* fenvalerate, 10^{-7} *M* permethrin, and 10^{-6} *M* cypermethrin treatment, respectively. After deltamethrin treatment, there is no significant effect.

DISCUSSION

E-Screen has been suggested as a method to screen large numbers of compounds quickly for estrogenicity. Cell proliferation is used as a hallmark of estrogen action, and assay theory assumes cell proliferation upon exposure to estrogenic compounds. Soto et al. (1995) exposed cells to 17β-estradiol for 6 d directly following overnight attachment. Jones et al. (1997) reported that addition to the protocol of a preincubation stage of cells in EMEM/H– (i.e., medium from which phenol and endogenous estrogens had been removed) for 48 h after attachment and prior to addition of 17β-estradiol for 6 d resulted in an approximately twofold increase in ER content of the MCF-7 cells from 24.6 to 54.2 fmol/mg protein and an increased maximum proliferation from 200–250 to 400% or more. In our study 24 h was the preincubation time, because in our preliminary experiment it was evident that 24-h and 48-h preincubation produced a similar effect on cell proliferation. There is concern that there are differences in the strain of MCF-7 cells, particularly in their proliferation response to the endogenous estrogen 17β-estradiol, thereby limiting usefulness to detect weak estrogens (Villalobos et al., 1995). Villalobos and coworkers (1995) indicated that MCF7BUS

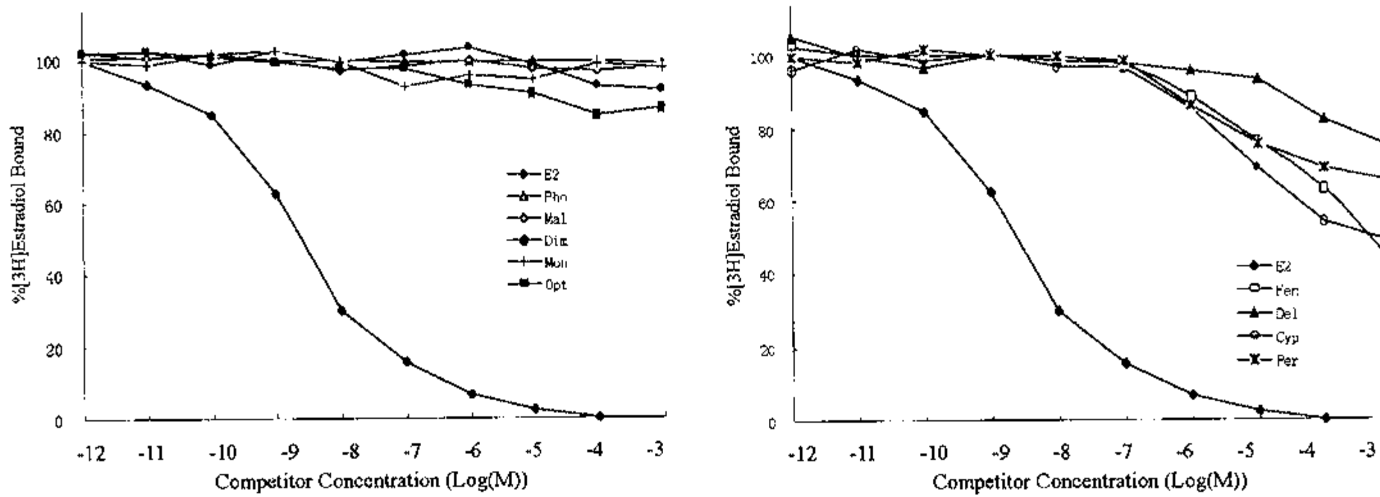

FIGURE 4. Estrogen-receptor binding affinity curves for the organophosphorus and pyrethroid pesticides.

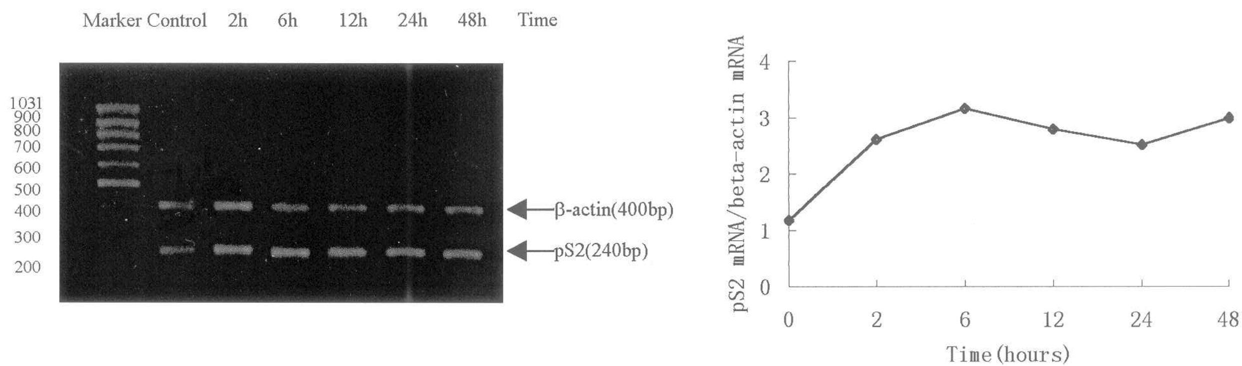

FIGURE 5. Time course of pS2 expression in MCF-7 cells after 17β-estradiol (0.1 μ*M*) treatments.

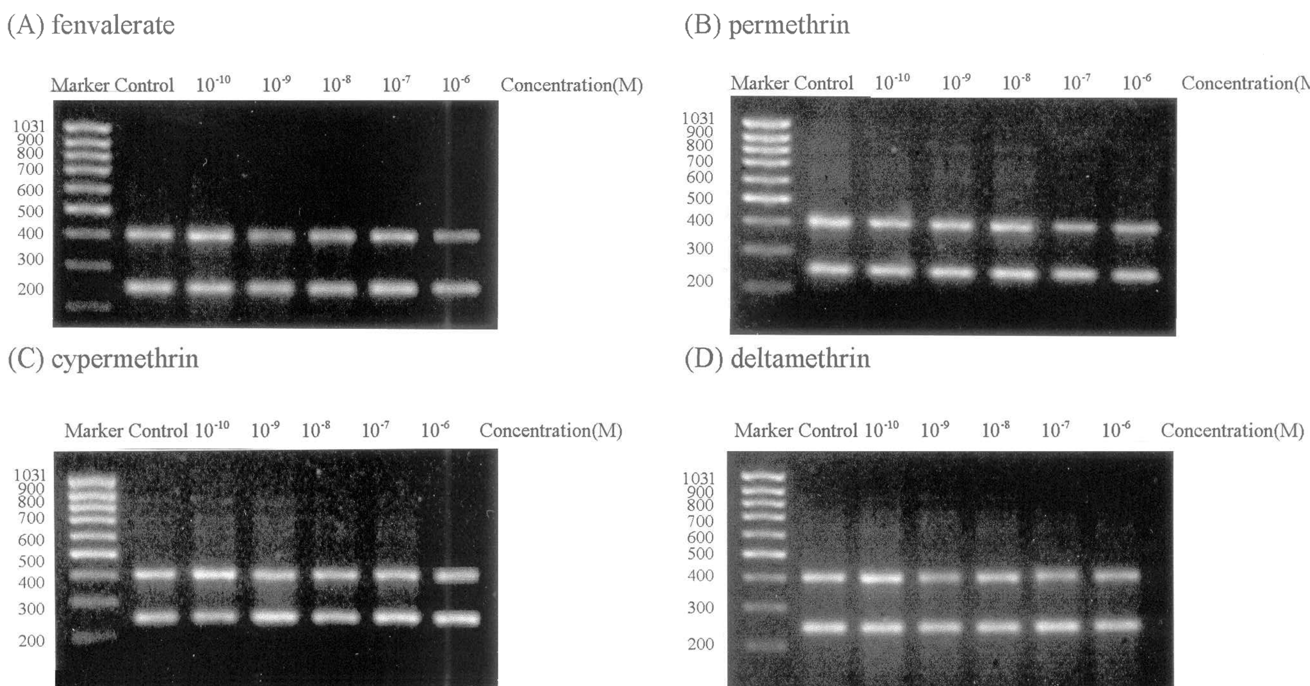

FIGURE 6. Concentration-dependent induction of pS2 expression in MCF-7 cells by pyrethroid pesticides for 6 h ($n = 3$). C, control (0.1% ethanol or DMSO). Results are presented as mean ± SD of three separate experiments. Asterisk indicates significantly different from control ($p < .05$).

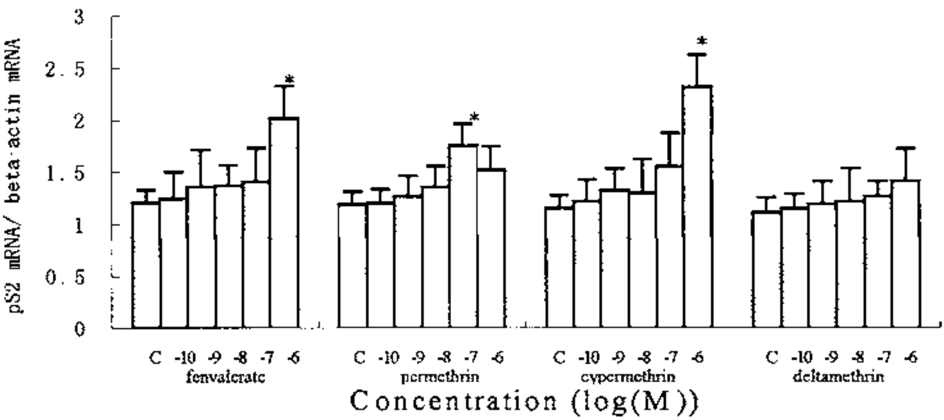

FIGURE 6. (*Continued*) Concentration-dependent induction of pS2 expression in MCF-7 cells by pyrethroid pesticides for 6 h ($n = 3$). C, control (0.1% ethanol or DMSO). Results are presented as mean ± SD of three separate experiments. Asterisk indicates significantly different from control ($p < .05$).

cells were the most responsive strain. However, those particular cells are not currently available in all laboratories. In our study, 1 n*M* 17β-estradiol induced 3.46-fold proliferation, indicating that MCF-7 cells used are sensitive to 17β-estradiol. It also showed that organophosphorus and pyrethroid pesticides exert different effects on cell proliferation. The latter increase MCF-7 cell proliferation, while the former produce no effect.

Typically, E-Screen assays involve serum that is stripped of steroidal components but still contains growth factors and metabolites. The potential for false negative and positive results increases when cross-talk is overlooked (Lackey et al., 2001). To study whether the effect on MCF-7 cell proliferation of the pesticides in E-Screen assay reflected the abilities of these chemicals to bind to ER, the effect of ER antagonist ICI 182.780 on pyrethroid pesticide-induced proliferation was determined. ICI 182.780 is a new steroidal antiestrogen that shows promise in current clinical trials of estradiol-induced breast cancer cells. ICI 182.780 belongs to the type II antiestrogens available for study (MacGregor & Jordan, 1998). What is unique about the type II antiestrogens is the observation that they produce the destruction of the ER in breast cancer cells in culture (Dauvois et al., 1992). ICI 182.780 binds to the newly synthesized receptor in the cytoplasm and prevents transport to the nucleus (Dauvois et al., 1993). In our study, ICI 182.780 was found to be a potent inhibitor of cypermethrin-, permethrin-, and deltamethrin-induced proliferation. The proliferation induced by fenvalerate could be only partly blocked. These results further support a role of ER in mediating pyrethroid pesticides-induced cell proliferation.

Chemicals may show ER activation through altering (1) ER conformation (without binding like estradiol), (2) ER phosphorylation status, (3) kinases or phosphatase activity, and (4) cofactor levels or availability (Harnagea-Theophilus et al., 1999). In an alternative, indirect pathway, estradiol upregulates some growth factors and their receptors, leading to an autocrine stimulation of proliferation (de Cupis & Favoni, 1997). The results from ER competitive-binding assay showed organophosphorus pesticides tested do not bind to the ER, while pyrethroid pesticides bind to the ER with different potency.

The human pS2 gene is approx. 4.5 kb long and contains 3 exons that encode the amino-terminal signal peptide, the trefoil (TFF) domain, and a carboxy-terminal acidic motif, respectively (Jeltsch et al., 1987). The promoter region contains an estradiol-responsive element (ERE) (Berry et al., 1989) and a complex-enhancer region responsive to epidermal growth factor (EGF), tumor promotor TPA, and the proto-oncoproteins c-Ha-ras and c-jun (Nunez et al., 1989). The regulation of the pS2 gene expression has been studied in breast carcinomas (Ribieras et al., 1998). The ectopic expression of pS2 is directly regulated by estradiol at the transcriptional level through the ERE (Rio et al., 1987). The pS2 gene product is a polypeptide that is expressed by one-half of all breast tumors, and although its function has not been determined, its presence identifies tumors that are sensitive to antihormonal therapy (Zacharewski et al., 1996). pS2-mRNA can be rapidly induced by estradiol in certain breast cancer biopsies (not by other steroid hormones such as progestins, glucocorticoids, or androgens), but not in normal breast cancer tissue or in any other cultured human cell lines. pS2 mRNA has also been sequenced. Therefore, pS2 mRNA expression in MCF-7 cells is an ideal model to study the effects of estrogenic chemicals on gene expression in human breast cancer (Ren et al., 1997). Using the expression of pS2 genes as estrogen-responsive reporter gene, the effects of estradiol and pyrethroid pesticides on MCF-7 were studied. In the time-course study, the pS2 mRNA expressions were detected after pesticides or estradiol treatments using the RT-PCR technique. The results indicated that some of the pyrethroid pesticides tested (fenvalerate, permethrin, and cypermethrin) induce pS2 expression in MCF-7 cell, while there are no significant effect after deltamethrin treatment. Compared with the E-Screen assay, pS2 mRNA expression is less sensitive to screen the estrogenicity of chemicals. While not a perfect test for endocrine disruptor screening, when examined in proper context, proliferation assays can provide useful data and be incorporated into a screening plan.

This study demonstrated estrogenic activity of pyrethroid pesticides tested. Recent studies showed antiandrogenic activity for environmental chemicals such as vinclozolin, a fungicide, and DDE, a insecticide (Sonnenschein & Soto, 1998). Moreover, a single chemical may produce neurotoxic, estrogenic, and antiandrogenic effects. Thus, pesticides may produce effects on endocrine and reproductive systems through other mechanisms, such as (1) mimicking the effect of endogenous hormones except for estrogen, (2) antagonizing the effect of endogenous hormones, (3) disrupting the synthesis and metabolism of endogenous hormones, and (4) disrupting the synthesis and metabolism of hormone receptors.

REFERENCES

Aprea, C., Sciarra, G., Sartorelli, P., Mancini, R., and Luca, V. D. 1998. Environmental and biological monitoring of exposure to mancozea, ethylenethiourea, and dimethoate during industrial formulation. *J. Toxicol. Environ. Health A* 53:263–281.

Abu-Qare, A. W., Abdel-Rahman, A., Brownie, C., Kishk, A. M., and Abou-Donia, M. B. 2001. Inhibition of cholinesterase enzymes following a single dermal dose of chlorpyrifos and methyl parathion, alone and in combination, in pregnant rats. *J. Toxicol. Environ. Health A* 63:173–189.

Berry, M., Nunez, A. M., and Chambon, P. 1989. Estrogen-responsive element of the human pS2 gene is an imperfectly palindromic sequence. *Proc. Natl. Acad. Sci. USA* 86:1218–1222.

Blair, R. M., Fang, H., Branham, W. S., Hass, B. S., Dial, S. L., Moland, C. L., Tong, W., Shi, L., Perkins, R., and Sheehan, D. M. 2000. The estrogen receptor relative binding affinities of 188 natural and xenochemicals: Structural diversity of ligands. *Toxicol Sci.* 54:138–153.

Carney, E. W., Hoberman, A. M., Farmer, D. R., Kapp, R. W., Jr., Nikiforov, A. I., Bernstein, M., Hurtt, M. E., Breslin, W. J., Cagen, S. Z., and Daston, G. P. 1997. Estrogen modulation: Tiered testing for human hazard evaluation. *Reprod. Toxicol.* 11:879–892.

Cole, D. C., Carpio, F., Julian, J., and Léon, N. 1998. Assessment of peripheral nerve function in an Ecuadorian rural population exposed to pesticides. *J. Toxicol. Environ. Health A* 55:77–91.

Dauvois, S., Danielian, P. S., White, R., and Parker, M. G. 1992. Antiestrogen ICI 164,384 reduces cellular estrogen receptor content by increasing its turnover. *Proc. Natl. Acad. Sci. USA* 89:4037–4041.

Dauvois, S., White, R., and Parker, M. G. 1993. The antiestrogen ICI 182780 disrupts estrogen receptor nucleocytoplasmic shuttling. *J. Cell. Sci.* 106:1377–1388.

de Cupis, A., and Favoni, R. E. 1997. Oestrogen/growth factor cross-talk in breast carcinoma: A specific target for novel antioestrogens. *Trends Pharmacol. Sci.* 18:245–251.

Garey, J., and Wolff, M. S. 1998. Estrogenic and antiprogestagenic activities of pyrethroid insecticides. *Biochem. Biophys. Res. Commun.* 251:855–859.

Gillesby, B. E., and Zacharewski, T. R. 1999. pS2(TFF1) levels in human breast cancer tumor samples: correlation with clinical and histological prognostic markers. *Breast Cancer Res. Treat.* 56:253–265.

Go, V., Gray, J., Wolff, M. S., and Pogo, B. G. 1999. Estrogenic potential of certain pyrethroid compounds in the MCF-7 human breast carcinoma cell line. *Environ. Health Perspect.* 107:173–177.

Gray, L. E., Jr., Kelce, W. R., Wiese, T., Tyl, R., Gaido, K., Cook, J., Klinefelter, G., Desaulniers, D., Wilson, E., Zacharewski, T., Waller, C., Foster, P., Laskey, J., Reel, J., Giesy, J., Laws, S., McLachlan, J., Breslin, W., Cooper, R., Di Giulio, R., Johnson, R., Purdy, R., Mihaich, E., Safe, S., and Colborn, T. 1997. Endocrine screening methods workshop report: Detection of estrogenic and androgenic hormonal and antihormonal activity for chemicals that act via receptor or steroidogenic enzyme mechanisms. *Reprod. Toxicol.* 11:719–750.

Harnagea-Theophilus, E., Gadd, S. L., Knight-Trent, A. H., DeGeore, G. L., and Miller, M. R. 1999. Acetaminophen-induced proliferation of breast cancer cells involves estrogen receptors. *Toxicol. Appl. Pharmacol.* 155:273–279.

Jeltsch, J. M., Roberts, M., Schatz, C., Garnier, J. M., Brown, A. M., and Chambon, P. 1987. Structure of the human oestrogen-responsive gene pS2. *Nucleic Acids Res.* 25(15):1401–1414.

Jones, P. A., Baker, V. A., Irwin, A., and Earl, L. K. 1997. Modulation of MCF-7 cell proliferative response by manipulation of assay conditions. *Toxicol. In Vitro* 11:769–773.

Jorgensen, M., Vendelbo, B., Skakkebaek, N. E., and Leffers, H. 2000. Assaying estrogenicity by quantitating the expression levels of endogenous estrogen-regulated genes. *Environ. Health Perpect.* 108:403–412.

Klotz, D. M., Arnold, S. F., and McLachlan, J. A. 1997. Inhibition of 17 beta-estradiol and progesterone activity in human breast and endometrial cancer cells by carbamate insecticides. *Life. Sci.* 60:1467–1475.

Lackey, R. R., Gray, S. I., and Henricks, D. M. 2001. Crosstalk and considerations in endocrine disruptor research. *Med. Hypoth.* 56:644–647.

Lowry, O. H., Rosebrough, N. L., Farr, A. L., and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265–275.

McLachlan, A. J. 1993. Functional toxicology: A new approach to detect biologically active xenobiotics. *Environ. Health Perspect.* 101:386–387.

MacGregor, J. I., and Jordan, V. C. 1998. Basic guide to the mechanisms of antiestrogen action. Basic guide to the mechanisms of antiestrogen action. *Pharmacol. Rev.* 50:151–196.

Mellanen, P., Petanen, T., Lehtimaki, J., Makela, S., Bylund, G., Holmbom, B., Mannila, E., Oikari, A., and Santti, R. 1996. Wood-derived estrogens: Studies in vitro with breast cancer cell lines and in vivo in trout. *Toxicol. Appl. Pharmacol.* 136:381–388.

Mileson, B. E., Chambers, J. E., Chen, W. L., Dettbarn, W., Ehrich, M., Eldefrawi, A. T., Gaylor, D. W., Hamernik, K., Hodgson, E., Karczmar, A. G., Padilla, S., Pope, C. N., Richardson, R. J., Saunders, D. R., Sheets, L. P., Sultatos, L. G., and Wallace, K. B. 1998. Common mechanism of toxicity: A case study of organophosphorus pesticides. *Toxicol. Sci.* 41:8–20.

Nunez, A. M., Berry, M., Imler, J. L., and Chambon, P. 1989. The 5′ flanking region of the pS2 gene contains a complex enhancer region responsive to oestrogens, epidermal growth factor, a tumour promoter (TPA), the c-Ha-ras oncoprotein and the c-jun protein. *EMBO J.* 8:823–829.

Petit, F., Le Goff, P., Cravedi, J. P., Valotaire, Y., and Pakdel, F. 1997. Two complementary bioassays for screening the estrogenic potency of xenobiotics: Recombinant yeast for trout estrogen receptor and trout hepatocyte cultures. *J. Mol. Endocrinol.* 19:321–335.

Pope, C. N. 1999. Organophosphorus pesticides: Do they all have the same mechanism of toxicity? *J. Toxicol. Environ. Health B* 2:161–181.

Rawlings, N. C., Cook, S. J., and Waldbing, D. 1998. Effects of the pesticides carbofuran, chlorpyrifos, dimethoate, lindane, triallate, trifluralin, 2,4-D, and pentachlorophenol on the metabolic endocrine and reproductive endocrine system in ewes. *J. Toxicol. Environ. Health A* 54:21–36.

Ren, L., Marquardt, M. A., and Lech, J. J. 1997. Estrogenic effects of nonylphenol on pS2, ER and MUC1 gene expression in human breast cancer cells-MCF-7. *Chem. Biol. Intract.* 104:55–64.

Ribieras, S., Tomasetto, C., and Rio, M. C. 1998. The pS2/TFF1 trefoil factor, from basic research to clinical applications. *Biochim. Biophys. Acta* 1378:F61–F77.

Rio, M. C., Bellocq, J. P., Gairard, B., Rasmussen, U. B., Krust, A., Koehl, C., Calderoli, H., Schiff, V., Renaud, R., and Chambon, P. 1987. Specific expression of the pS2 gene in subclasses of breast cancers in comparison with expression of the estrogen and progesterone receptors and the oncogene ERBB2. *Proc. Natl. Acad. Sci. USA* 84:9243–9247.

Roy, D., Palangat, M., Chen, C. W., Thomas, R. D., Colerangle, J., Atkinson, A., and Yan, Z. J. 1997. Biological and molecular changes at the cellular level in response to exposure to environmental estrogen-like chemicals. *J. Toxicol. Environ. Health* 50:1–29.

Saito, K., Tomigahara, Y., Ohe, N., Isobe, N., Nakasuka, I., and Kaneko, H. 2000. Lack of significant estrogenic or antiestrogenic activity of pyrethroid insecticides in three in vitro assays based on classic estrogen receptor alpha-mediated mechanisms. *Toxicol. Sci.* 57:54–60.

Sonnenschein, C., and Soto, A. M. 1998. An updated review of environmental estrogen and androgen mimics and antagonists. *J. Steroid. Biochem. Mol. Biol.* 65:143–150.

Soto, A. M., Sonnenschein, C., Chung, K. L., Fernandez, M. F., Olea, N., and Serrano, F. O. 1995. The ESCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. *Environ. Health Perspect.* 103(S7):113–122.

Tonilol, P. G., Levitz, M., Zeleniuch, J. A., Banerjee, S., Koenig, K. L., Shore, R. E., Strax, P., and Pasternack, B. S. 1995. A prospective study of endogenous estrogens and breast cancer in postmenopausal women. *JNCI* 87:190–197.

Villalobos, M., Olea, N., Brotons, J. A., Olea-Serrano, M. F., Ruiz, d. A. J. M., and Pedraza, V. 1995. The E-screen assay: A comparison of different MCF7 cell stocks. *Environ. Health Perspect.* 103:844–850.

Wang, F., Porter, W., Xing, W., Archer, T. K., and Safe, S. 1997. Identification of a functional imperfect estrogen-responsive element in the 5′-promoter region of the human cathepsin D gene. *Biochemistry* 36:7793–7801.

Zacharewski, T. R., Bondy, K. L., McDonell, P., and Wu, Z. F. 1996. Antiestrogenic effect of 2, 3, 7,8-tetrachlorodibenzo-*p*-dioxin on 17β-estradiol-induced pS2 expression. *Cancer Res.* 54:2707–2713.